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MINIREVIEW

Cellular Factors in the Transcription and Replication of Viral RNA Genomes: A Parallel to DNA-Dependent RNA Transcription

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Viral RNA replication and transcription involves not only viral RNA-dependent RNA polymerases, but also cellular proteins, the majority of which are subverted from the RNA-processing or translation machineries of host cells. These factors interact with viral RNA or polymerases to form transcription or replication ribonucleoprotein complexes and may provide template specificity for RNA-dependent RNA synthesis, suggesting a close parallel to the mechanism of DNA-dependent RNA synthesis. The types of cellular proteins involved and their modes of action are reviewed.

Because viruses contain only limited genetic information, they must rely on existing or modified cellular machineries for many steps of macromolecular synthesis. This is true not only of protein translation, but also of the replication and transcription of viral DNA genomes. For example, gene expression of most DNA viruses is effected by cellular polymerases and regulated largely by cellular transcription factors. In contrast, the participation of cellular factors in the transcription and replication of viral RNA genomes is less obvious in RNA viruses. At first glance, this uncertain role of cell factors appears to be logical since most RNA viruses replicate and transcribe their genomes by RNA-dependent RNA synthesis, a process foreign to most eukaryotic cells. Thus, it is assumed that normal cells do not have preexisting machinery to support viral RNA-dependent RNA synthesis and that RNA viruses are more self-reliant, depending on their own viral proteins for RNA replication and transcription. This view is consistent with the fact that most RNA viruses shut off cellular transcription and translation for the purpose of viral replication. It is, however, paradoxical because RNA viruses typically have a small genome; thus, we would intuitively assume that they must rely more on the cell to assist in viral replication. Indeed, there is increasing evidence that RNA viruses frequently subvert cellular factors for replication and transcription of viral RNAs. Many of these factors are normal components of cellular RNA processing or translation machineries, which are subverted to play an integral or regulatory role in the replication and transcription of viral RNA. This revised view is consistent with the long-recognized observation that replication of many RNA viruses and their mutants is cell type-specific, suggesting their dependence on cell-specific factors (e.g., Kowal and Stollar, 1981; Kuhn *et al.,* 1981; Shiroki *et al.,* 1993). The most compelling evidence for the participation of cellular factors in viral RNA synthesis came from a heterologous system that studied brome mosaic virus (BMV) replication in yeast (Janda and Ahlquist, 1993). Multiple yeast mutants that affect BMV RNA replication or transcription have been isolated (Ishikawa *et al.,* 1997). Therefore, it now appears that cellular factors are surprisingly active in the replication and transcription of viral RNAs, even though cells are not equipped to carry out RNA-dependent RNA synthesis on their own. This review will summarize the existing evidence and provide perspectives on the roles of the cellular factors that have been proven or implicated to be directly involved in viral RNA synthesis. Many other cellular factors, such as protein kinases or other protein-modifying enzymes, may affect viral RNA synthesis indirectly by modulating the properties or biosynthesis of viral proteins. This latter category of cellular factors will not be discussed. Readers should also refer to two recent reviews by Buck (1996) and De and Banerjee (1997), which provide comprehensive descriptions of the individual viruses and cellular proteins discussed here.

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Purified viral RNA polymerases without cellular proteins are usually enzymatically inactive or nonselective for template

Prokaryotic and eukaryotic DNA-dependent RNA polymerases usually consist of multiple subunits. Typically, the core enzymes do not confer template specificity. The specificity of RNA synthesis is usually determined by other factors, such as bacterial or bacteriophage sigma factors or mammalian transcription factors, which are either tightly associated with the polymerase holoenzyme or exist independently of the polymerases but interact with the template. Similarly, relatively pure preparations of viral RNA-dependent RNA polymerase (RdRP) from several viruses, such as poliovirus (Neufeld *et al.,* 1991), hepatitis C virus (Behrens *et al.,* 1996), or dengue virus (Tan *et al.,* 1996), can replicate most natural or synthetic RNA or even DNA templates *in vitro* with very little template specificity (which usually requires a primer or is self-priming, in contrast to viral RNA synthesis *in vivo,* which is usually primer-free). Only a few viral RdRPs, e.g., those of cucumber mosaic virus (CMV) (Hayes and Buck, 1990), brome mosaic virus (BMV) (Quadt *et al.,* 1993; Siegel *et al.,* 1997), and turnip crinkle virus (TCV) (Song and Simon, 1994), have been shown to replicate or transcribe viral RNAs specifically; however, all of the RdRP preparations purified from virus-infected cells (for positive-strand RNA viruses) contained some cellular proteins. Removal of the cellular factors invariably resulted in the loss of RdRP activity or template specificity. Thus, it appears that the core viral polymerase proteins, at best, carry only the basic RdRP activity but not the determinant of their template specificity. In negative-strand RNA viruses, RdRPs are typically present in the virion; disruption of the virion leads to transcription of viral RNA under *in vitro* reaction conditions. Even under these conditions, viral replicase and its associated viral proteins (typically nucleocapsid protein and some other proteins, e.g., phosphoprotein) are not sufficient to replicate or transcribe virion RNA. Instead, cellular factors, such as tubulin, actin, and heat-shock proteins, are required for RdRP reactions (De *et al.,* 1991; Huang *et al.,* 1993; Moyer *et al.,* 1986; Oglesbee *et al.,* 1996). In the poliovirus translation–replication-coupled system, initiation of RNA synthesis from a preinitiation complex also requires a cellular factor (Barton *et al.,* 1995). All these observations suggest that cellular factors are necessary for template-specific RNA-dependent RNA synthesis, which is undoubtedly a prerequisite for successful viral replication, considering the fact that viral RdRPs have to replicate only viral RNA but not numerous cellular RNAs present in the infected cells.

Cellular proteins as components of RdRP

The participation of cellular factors in viral RNA-dependent RNA synthesis follows two modes (Table 1): In the first, cellular proteins are present as part of the RdRP holoenzyme; in the second, they bind directly to the RNA template, directing RdRP to the template. However, these two modes are not mutually exclusive. In fact, some factors may possess both functions and thus serve as a bridge between viral RdRPs and viral RNA template. The classification of these two modes of action may merely reflect the degree of difference in the affinity of a factor for either RdRP or RNA and the method of detection used by the investigators. The classical evidence for the first mode is provided by the Qβ RdRP holoenzyme, which contains at least three host proteins, EF-Tu, EF-Ts (equivalents of mammalian translation factor EF-1 subunits), and ribosomal protein S1, in addition to the phage-encoded replicase (Blumenthal and Carmichael, 1979; van Duin, 1988). Removal of these cellular factors resulted in the complete loss of Qβ replicase activity. The RdRP holoenzyme can direct the synthesis of positive-strand RNA from the template (negative-strand) RNA of Qβ phage, but does not direct the synthesis of negative-strand RNA from the phage genome unless another cellular factor HF1 (also a ribosome-associated protein) binds to the 3′ end of the phage genomic RNA (Barrera *et al.,* 1993). Thus, cellular factors play dual roles at two different steps of Qβ phage RNA synthesis: It is an integral part of the RdRP holoenzyme for positive-strand RNA synthesis, and it binds directly to the template RNA for negative-strand RNA synthesis. In the first mode, the Qβ replicase resembles prokaryotic RNA polymerases, which contain a sigma factor in the holoenzyme as a determinant of template specificity (Helmann and Chamberlin, 1988). In the second mode, it shows a close parallel to eukaryotic DNA-dependent RNA polymerase II, which, by itself, does not bind to transcriptional promoters, but is recruited to the promoters via protein–protein interactions with the TATA-binding protein (TBP) and other general transcription factors. It is interesting to note that all the host factors associated with Qβ RdRP are components of the cellular translation machinery, suggesting a possible linkage between translation and viral RNA synthesis. There is some evidence that translation and replication of viral RNAs may be coupled, as reported for poliovirus (Molla *et al.,* 1991; Novak and Kirkegaard, 1994) and other viruses (e.g., White *et al.,* 1992); however, this is not universally true. Alternatively, the presence of the translation factors in the Qβ replicase may simply suggest that components of cellular translational machinery are subverted for viral RNA synthesis.

A second, well-characterized cellular factor in the viral RdRP preparations is the eukaryotic translation initiation factor eIF-3, which is present in BMV and tobacco mosaic virus (TMV) RdRPs (Osman and Buck, 1997; Quadt *et al.,* 1993). eIF-3 was found to be necessary for the RdRP activity of both viruses. In the case of BMV, it binds directly to viral polymerase protein (2a protein), but its

TABLE 1

Modes of Action of Cellular Proteins Involved in Viral RNA-Dependent RNA Synthesis

			References
I. Associated with RdRP			
Qβ phage		EF-Tu, Ts (EF-1), ribosomal protein S1	Blumenthal and Carmichael (1979)
Brome mosaic virus		eIF-3	Quadt *et al.* (1993)
Tobacco mosaic virus		eIF-3	Osman and Buck (1997)
Vesicular stomatitis virus		EF-1α, β, γ	Das *et al.* (1998)
Measles virus		Tubulin	Moyer *et al.* (1990)
Poliovirus		EF-1α	Harris *et al.* (1994)
		Sam68	McBride *et al.* (1996)
II. Binding to viral RNA			
Qβ phage	3′-UTR (+)	HF1 (ribosomal protein)	Barrera *et al.* (1993)
Poliovirus	5′-UTR (+)	PTB	Hellen *et al.* (1993)
		La	Meerovitch *et al.* (1993)
		Poly(rC)-binding protein	Parsley *et al.* (1997); Gamarnik and Andino (1997)
Sindbis virus	3′-UTR (−)	La	Pardigon and Strauss (1996)
Mouse hepatitis virus	3′-leader (−), IG (−)	hnRNP A1	Li *et al.* (1997)
	5′-leader (+)	PTB	Li and Lai (unpublished)
Hepatitis C virus	3′-UTR (+) 5′-UTR (+)	PTB	Ito and Lai (1997); Ali and Siddiqui (1997); Tsuchihara *et al.* (1997)
Hepatitis A virus	5′-UTR (+)	GAPDH, PTB	Schultz *et al.* (1996)
West Nile virus	3′-UTR (+)	EF-1α	Blackwell and Brinton (1997)
Turnip yellow mosaic virus	3′-UTR (+)	EF-1α	Joshi *et al.* (1986)
Vesicular stomatitis virus	Leader RNA (+) (−)	La	Kurilla and Keene (1983); Wilusz *et al.* (1983)
Rubella virus	3′-UTR (+)	Calreticulin	Singh *et al.* (1994)
	5′-UTR (+)	La	Pogue *et al.* (1996)
Human parainfluenza virus-3	3′-UTR (+) (−)	La, GAPDH	De *et al.* (1996)
Human immunodeficiency virus	5′-UTR (TAR) (+)	La	Chang *et al.* (1994); Svitkin *et al.* (1994)
	5′-LTR/CRS	PTB, hnRNP A1	Black *et al.* (1996)
Human T-cell leukemia virus-2	5′-LTR/CRS	PTB, hnRNP A1	Black *et al.* (1995)
III. Undefined factors (cytoskeletal or chaperone proteins)			
Vesicular stomatitis virus		Tubulin	Moyer *et al.* (1986)
Sendai virus		Tubulin	Moyer *et al.* (1986)
Respiratory syncytial virus		Tubulin	Huang *et al.* (1993)
Human parainfluenza virus-3		Actin	De *et al.* (1991)
Canine distemper virus		Heat-shock protein	Oglesbee *et al.* (1996)

Note. Abbreviations: UTR, untranslated region. CRS, *cis*-acting repressive sequence. HR1, host factor-1. PTB, polypyrimidine tract-binding protein. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. IG, intergenic sequence. (+) and (−) represent positive- and negative-strand RNA, respectively.

mode of action in the TMV RdRP is not yet clear. Interestingly, although BMV and TMV RdRPs both contain eIF-3, which consists of 10 subunits, they actually contain different subunits of eIF-3, suggesting that the functional or mechanistic roles of this translation factor in these two viruses are different. Nevertheless, the fact that both are subunits of the same translation factor is striking.

Purified, enzymatically active poliovirus RdRP (virion protein 3CD) has been shown to contain several host proteins, one of which is EF-1α (Harris *et al.,* 1994). The identity of the other factors is less certain: one may be poly(rC)-binding protein (hnRNP E), which, together with 3CD protein, binds directly to the 5′ end of poliovirus RNA (Gamarnik and Andino, 1997; Parsley *et al.,* 1997). These factors appear to be involved in positive-strand RNA synthesis. Other factors have been reported to be involved in negative-strand RNA synthesis (Dasgupta *et al.,* 1980). The poliovirus RdRP also binds a nuclear protein, Sam68, which is involved in cellular mitosis, *in vitro* (McBride *et al.,* 1996). This protein relocalizes to the cytoplasm in the poliovirus-infected cells, where the virus replicates, suggesting that this binding is of functional significance in poliovirus infection. However, it has not been established that this protein is directly involved in poliovirus RNA replication, and Sam68 has not been found to be associated with the purified viral RdRP.

The purified vesicular stomatitis virus (VSV) RdRP (L protein) also requires the addition of cellular factors for its enzymatic activity *in vitro.* These factors have recently been identified as the eukaryotic translation factor EF-1β and γ subunits (Das *et al.,* 1998). Interestingly, the purified L protein itself contains a tightly associated EF-1α subunit (Das *et al.,* 1998). Thus, all three subunits of EF-1 are associated either directly or indirectly with VSV RdRP. These findings again suggest the potential linkage between the cellular translation machinery and viral RNA

synthesis. Other cellular proteins detected in the various viral RdRP preparations include the protein in the CMV RdRP (Hayes and Buck, 1990), which has been shown to be required for RdRP enzymatic activity, but whose identity has not been determined. Many other reported viral RdRP preparations, e.g., those from cowpea mosaic virus (Dorssers *et al.,* 1984), red clover necrotic mosaic virus (Bates *et al.,* 1995), and Sindbis virus (Barton *et al.,* 1991), contain various cellular proteins. However, the purity of these preparations has not been established; therefore, whether these proteins are essential components of RdRPs or merely contaminants is not clear. Influenza virus NP protein, which is involved in viral RNA synthesis, also binds to heterogeneous nuclear ribonucleoprotein hnRNP C1 (O'Neill and Palese, 1994). The functional significance of this binding is not yet known.

Cellular proteins interacting with the *cis*-acting regulatory viral RNA sequences for RNA synthesis

The second mechanism by which cellular proteins participate in viral RNA synthesis is by binding to viral RNA. The classic example is the binding of the host factor to the 3′ end of Qβ phage RNA, which is required for minus-strand RNA synthesis (Barrera *et al.,* 1993). An increasing number of cellular proteins have now been shown to bind to viral RNA regions that regulate RNA synthesis of various viruses. These regions include the 5′ and 3′ ends and internal promoters of the various viral genomes and antigenomes. The proteins were typically detected by *in vitro* UV crosslinking or gel mobility shift experiments using viral RNA and uninfected cell lysates. In some cases, these protein–RNA interactions were also demonstrated in virus-infected cells, and their formations correlated with viral RNA synthesis (Andino *et al.,* 1993; Chen *et al.,* 1997; Roehl and Semler, 1995). These viral RNA–protein complexes frequently contain both viral (polymerase) and cellular proteins. It should be noted that, although some purified viral polymerase proteins alone, e.g., those of poliovirus (Harris *et al.,* 1994) and encephalomyocarditis virus (EMCV) (Cui *et al.,* 1993), can preferentially bind homologous viral RNA at the regions required for viral RNA synthesis (e.g., 5′ and 3′ ends), their binding specificity and affinity is probably not sufficient to account for the specificity of viral RNA synthesis. In fact, many other viral RdRPs do not bind to viral RNA specifically or at all; thus, their binding to template RNA must be mediated by cellular proteins that bind specifically to viral RNA. Table 1 summarizes the various cellular proteins that have been found to be capable of binding to viral RNAs [with the exclusion of those proteins that bind to regions known only to regulate translation, e.g., internal ribosome entry site (IRES) of EMCV and foot-and-mouth-disease virus]. Many other proteins have been determined by UV cross-linking experiments to bind to various viral RNAs, but have not yet been characterized (these are not listed in Table 1).

Several proteins are of particular interest: (1) poly(rC)-binding protein (also known as hnRNP E) binds to both the 5′ end cloverleaf structure (Gamarnik and Andino, 1997; Parsley *et al.,* 1997), which is required for viral RNA replication, and the IRES (Blyn *et al.,* 1996), which is required for translation of poliovirus RNA. This protein forms a complex with viral protein 3CD (RdRP) and probably brings the RdRP to the 5′ end of the poliovirus RNA; however, the binding sites for 3CD and poly(rC)-binding protein are in different parts of the cloverleaf structure (Gamarnik and Andino, 1997). Conceivably, the binding of poly(rC)-binding protein alters the RNA conformation to facilitate 3CD binding and initiates RNA synthesis. Since this same protein binds to both the cloverleaf structure and IRES, which also bind other proteins (PTB and La) (Table 1), it is possible that all of these proteins interact, bringing these RNA regions together to form an RNA–protein complex. Thus, the binding of this protein to the 5′ end of the viral RNA may affect both the translation and RNA replication of poliovirus, again suggesting a close association between these two processes. (2) La antigen binds to many viral RNAs, including the 5′ end IRES of poliovirus RNA, the 3′ end of the negative-strand RNA of Sindbis virus, and the plus and minus strands of the leader RNAs of VSV (Kurilla and Keene, 1983; Wilusz *et al.,* 1983) and rabies virus (Kurilla *et al.,* 1984). La antigen binding is necessary for poliovirus RNA translation (Meerovitch *et al.,* 1993) and may also regulate RNA replication. It is probably required for RNA synthesis in Sindbis virus (Pardigon and Strauss, 1996), although this possibility was inferred from indirect evidence only. The function of the La binding to the VSV leader RNA is not clear; presumably, it modulates the function of the leader RNA in inhibiting host macromolecular synthesis and regulating the switch between viral RNA transcription and replication, but these functions have not been demonstrated. Interestingly, La also binds to the 5′-UTR of HIV RNA in the TAR region (*tat*-binding site) (Chang *et al.,* 1994; Svitkin *et al.,* 1994). It is but one of the many cellular proteins found to bind to this RNA sequence; presumably, these proteins together regulate the switching between transcription and translation of HIV RNA. (3) Polypyrimidine tract-binding protein (PTB; hnRNP I) also binds to many viral RNAs at several different sites, such as the IRES of poliovirus, hepatitis A (HAV), and hepatitis C viruses (HCV) (Ali and Siddiqui, 1997; Hellen *et al.,* 1993; Schultz *et al.,* 1996). In these cases, PTB has been shown to regulate translation. It also binds to the 3′ end of the HCV RNA at two conserved stem-loop structures (Ito and Lai, 1997; Tsuchihara *et al.,* 1997) and the 5′ end of mouse hepatitis virus (a coronavirus) RNA at a stretch of UCUAA repeats, which is critical for mRNA transcription (Li and Lai, unpublished observations). In these cases, PTB may regulate not only translation, but also

viral RNA synthesis. (4) hnRNP A1 binds to the leader sequence and mRNA transcription-initiation sites on the minus strand (template strand) of the MHV RNA (Li *et al.,* 1997). These sequences are important for the regulation of mRNA transcription. Site-specific mutagenesis of these sequences has shown a correlation between the extent of hnRNP A1 binding and the efficiency of transcription from these sites, suggesting that this protein is a requisite transcription factor (Zhang and Lai, 1995). hnRNP A1, together with PTB, also binds to the LTR of HIV and HTLV RNA at a region termed *cis*-acting repressive sequence (CRS), which modulates viral RNA splicing (Black *et al.,* 1995, 1996). The binding of both hnRNP A1 and PTB to this region may mediate the functions of the viral *rev* protein, thereby regulating viral RNA processing. It is significant that hnRNP A1 forms a dimer and also interacts with PTB, potentially bringing together all the RNA regulatory elements to form a transcription complex. In fact, hnRNP A1 and PTB were initially purified together as a pre-mRNA splicing complex in normal cells (Bothwell *et al.,* 1991). (5) Translation factors, such as EF-1α and HF1, bind to the 3′ ends of turnip yellow mosaic virus (TYMV), West Nile virus (WNV), and Qβ phage RNAs (Joshi *et al.,* 1986; Blackwell and Brinton, 1997; Barrera *et al.,* 1993). Translation factors are of particular interest because they not only bind to viral RNA, but some of them, e.g., EF-1α, β, γ, eIF-3, EF-Tu, and EF-Ts, are tightly associated with RdRPs of various viruses. Thus, translation factors may participate in viral RNA synthesis by either interacting directly with viral RNA or associating with RdRP. (6) Other proteins, including GAPDH and calreticulin, bind to viral RNAs, such as those of HAV and HPIV-3 (Schultz *et al.,* 1996; De *et al.,* 1996) and rubella virus (Singh *et al.,* 1994). Although GAPDH is known to be a housekeeping enzyme, it also has an RNA helix-destabilizing activity. Calreticulin may have direct gene-regulatory functions, besides being a calcium-binding protein. Thus, their binding to viral RNAs is consistent with their biological properties.

One striking feature of these RNA-binding proteins is that many of them can bind to several different, unrelated viral RNAs. La antigen, PTB, and EF-1α all have this ability. The binding specificity of each of these proteins for each viral RNA has been well established. Considering the fact that the RNAs in question do not share a primary sequence, the overlap among the RNA-binding protein profiles of the various viruses is surprising. This observation has two implications: (1) The RNA-binding specificity of these cellular proteins is more flexible than commonly realized, and these RNAs may share unrecognized structural features common among the viral RNA sequences that regulate RNA synthesis; and (2) some of these proteins bind to viral RNA in one virus but associate with viral polymerase in another. Thus, repeated detection of these proteins cannot be attributed solely to nonspecific RNA–protein interactions in all cases, but most likely reflects the real possibility that these cellular proteins are associated with viral RNA synthetic machinery.

TABLE 2

Types of Cellular Factors Involved in Viral RNA Synthesis

I. Heterogeneous nuclear ribonucleoprotein (hnRNP)	
hnRNP A1	MHV, HIV, HTLV-2
hnRNP I (PTB)	PV, MHV, HCV, HAV, HIV, HTLV-2
hnRNP E (poly rC-binding protein)	PV
hnRNP C1	Influenza virus
II. Pol III transcript-binding proteins	
La antigen	Sindbis virus, VSV, HIV, HPIV-3, PV, RV
III. Translation factors	
EF-1α	WNV, Qβ, VSV, PV, TYMV
EF-1β, γ	Qβ, VSV
eIF-3	BMV, TMV
Ribosomal protein	Qβ
IV. Cytoskeletal or chaperone protein	
Tubulin	VSV, Sendai virus, RSV
Actin	HPIV-3
Heat shock protein	CDV
V. Miscellaneous	
GAPDH	HAV, HPIV-3
Calreticulin	RV
Sam68	PV

Note. Abbreviations: MHV, mouse hepatitis virus. HIV, human immunodeficiency virus. HTLV-2, human T-cell leukemia virus-2. PV, poliovirus. HCV, hepatitis C virus. HAV, hepatitis A virus. VSV, vesicular stomatitis virus. HPIV-3, human parainfluenza virus-3. RV, rubella virus. WNV, West Nile virus. TYMV, turnip yellow mosaic virus. BMV, brome mosaic virus. TMV, tobacco mosaic virus. RSV, respiratory syncytial virus. CDV, canine distemper virus.

Cytoskeletal and chaperone proteins in viral RdRP reactions

Several paramyxoviruses and rhabdoviruses require other types of cellular factors for RNA synthesis in *in vitro* reactions. These include tubulin for VSV and Sendai viruses (Moyer *et al.,* 1986), measles virus (Moyer *et al.,* 1990), and respiratory syncytial virus (Huang *et al.,* 1993); actin for human parainfluenza virus 3 (De *et al.,* 1991); and heat shock protein for canine distemper virus (Oglesbee *et al.,* 1996). Tubulin is tightly associated with the purified RdRP complex of measles virus (Moyer *et al.,* 1986); the modes of action of the other proteins are not clear (although actin has been consistently detected in the virions of many paramyxoviruses). The precise functions of these proteins in viral RNA synthesis have not been established. One possibility is that they serve a structural function, holding the various components of RNA transcription or replication complex in correct topology. Indeed, the viral RNA–protein (RNP) complex of some paramyxoviruses, e.g., Newcastle disease virus, has been shown to be associated with the cellular cy-

toskeleton (Hamaguchi *et al.,* 1985). Many other viruses (not limited to paramyxoviruses) also synthesize their RNAs on or in discrete subcellular compartments, such as the endoplasmic reticulum (BMV) (Restrepo-Hartwig and Ahlquist, 1996) or membranous vesicles (poliovirus) (Egger *et al.,* 1996; Schlegel *et al.,* 1996). Cellular RNAs may be excluded from such restricted compartments, thus allowing viral RNA to be exclusively synthesized, providing an alternative mechanism to confer template specificity for viral RdRP. Interestingly, a poliovirus mutant lacking the entire 3′ end of its RNA genome, which comprises the *cis*-acting signal for RNA replication, is viable (Todd *et al.,* 1997). The phenotype of this mutant appears to be consistent with the interpretation that the template specificity of viral RdRP may not strictly depend on its recognition of specific *cis*-acting signals at the 3′ end of viral RNA but rather on the ability of the viral RNA and RdRP to be localized in the specialized subcellular compartments. Actin, tubulin, heat shock proteins, and other chaperone proteins may provide the means to transport or orient RNA and RdRPs in proper topology in these compartments. In this regard, it is significant to note that heat shock proteins are required for the reverse transcriptase activity of hepatitis B virus (Hu and Seeger, 1996) and bind to the capsid protein of poliovirus (Macejak and Sarnow, 1992). These arguments notwithstanding, it cannot be ruled out that these cytoskeletal and chaperone proteins may also have a more direct role in viral RNA synthesis; for example, tubulin can replace the acidic domain of the viral P protein of VSV in transcription *in vitro* (Chattopadhyay and Banerjee, 1988).

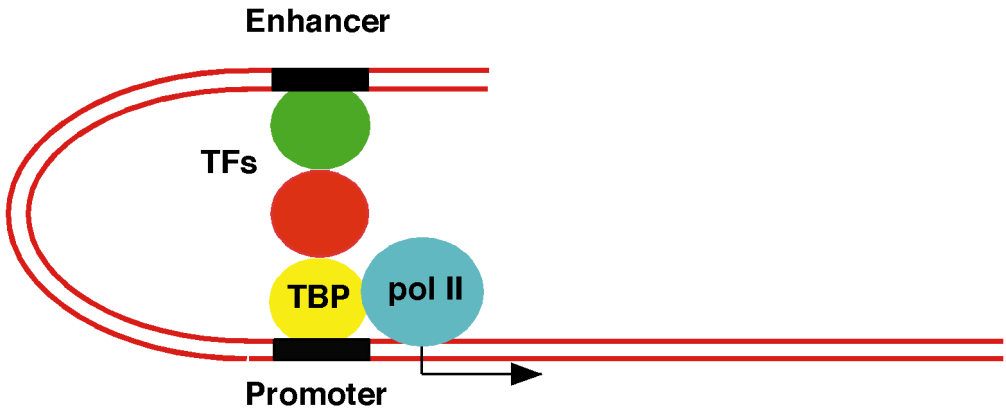
Types of cellular proteins involved in viral RNA synthesis

Examination of the cellular proteins discussed above revealed that they belong to five general classes, most of which are associated with RNA-processing pathways or translation machinery (Table 2): (1) hnRNP complex. This class is the most frequently observed. It includes hnRNP A1, PTB (hnRNP I), poly(rC)-binding protein (hnRNP E), and, possibly, hnRNP C1. These proteins are involved in cellular RNA transport and/or alternative splicing in normal cells. They have both RNA- and protein-binding activities. In RNA viruses, they have been shown to be involved in translation (e.g., PTB) and RNA synthesis [e.g., hnRNP A1 and poly(rC)-binding protein]. (2) Pol III transcript-binding protein. La antigen interacts with a significant number of viral RNAs at the *cis*-acting signals for RNA synthesis and IRES regions. This protein has also been implicated in translation (in picornaviruses) and RNA synthesis (in Sindbis virus and poliovirus) of various viruses. (3) Translation factors. Proteins of this class either bind directly to the viral RNA (WNV and TYMV) or complex with the components of the viral RdRP (Qβ replicase, BMV, TMV, VSV, and poliovirus polymerases). Paradoxically, translation factors are not involved in the regulation of translation of RNA viruses, since they do not bind to viral sequences directly involved in translation. Instead, they all interact with either RdRP or the *cis*-acting signals for RNA replication, suggesting their roles in viral RNA synthesis. It is noteworthy that many translation factors bind cellular RNAs in normal cells and have RNA helicase activities. These activities are compatible with the putative roles of the translation factors in viral RNA synthesis. (4) Cytoskeletal or chaperone proteins. These proteins participate prominently in the RNA synthesis of negative-strand RNA viruses. As discussed above, they may play structural roles in viral RNA synthesis. (5) Miscellaneous factors, including GAPDH, calreticulin, and Sam68. These proteins are not normally thought of as RNA-binding proteins. However, they have been shown to bind RNA under various conditions.

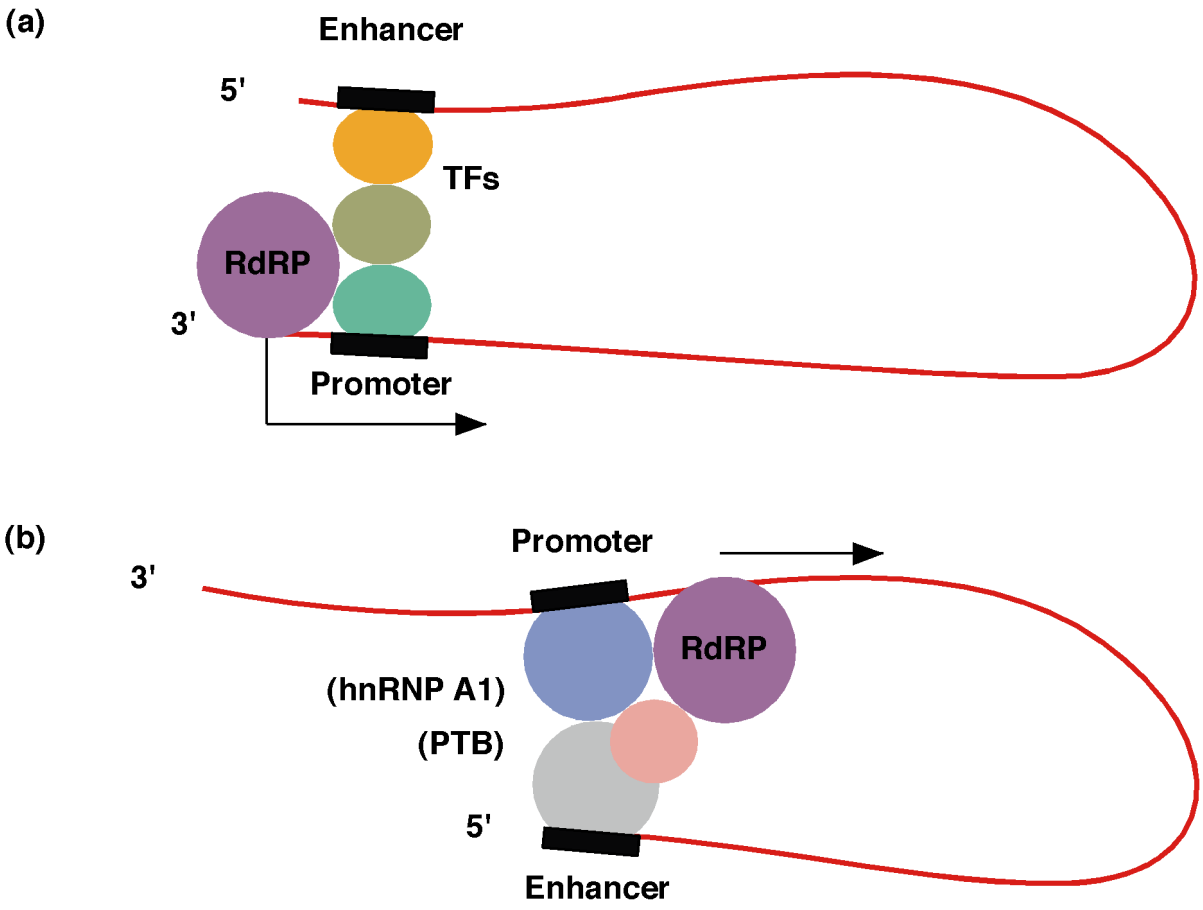
One notable feature of these proteins, with the exception of the translation factors, is that they are located predominantly in the nucleus of normal cells, whereas most RNA viruses replicate exclusively in the cytoplasm. This apparent contradiction may cast doubt on the biological significance of these cellular factors in viral RNA synthesis; however, some of these proteins may be shuttled between cytoplasm and nucleus (e.g., hnRNP A1 and PTB) and, certainly, all are synthesized in the cytoplasm. Thus, the association of these proteins with cytoplasmic viruses should not pose a conceptual difficulty. Indeed, the relocalization of these cellular proteins from nucleus to cytoplasm in virus-infected cells has been demonstrated for several proteins (La, hnRNP A1, PTB) (Li *et al.,* 1997; McBride *et al.,* 1996; Meerovitch *et al.,* 1993). It should be pointed out that several RNA viruses, e.g., poliovirus and MHV, have been shown to replicate in enucleated cells (Brayton *et al.,* 1981; Wilhelmsen *et al.,* 1981), leading to the conclusion that nuclear functions are not required for replication of these viruses. However, reexamination of these published data revealed that virus titers produced from the enucleated cells were usually significantly lower than that from the whole cells. Thus, the potential roles of cellular nuclei in RNA virus replication should be reexamined. It is possible that,

FIG. 1. Comparison of models of DNA- and RNA-dependent RNA synthesis. The colored circles represent transcription factors (TFs). In RNA-dependent RNA synthesis, RNA initiates from either one end (a) or an internal promoter (b). The putative enhancer and promoter are held together through transcription factors (cellular proteins). An example of such a pair of interacting cellular factors (hnRNP A1 and PTB for mouse hepatitis virus) is shown in (b).

DNA-DEPENDENT RNA TRANSCRIPTION



RNA-DEPENDENT RNA TRANSCRIPTION AND REPLICATION



even in enucleated cells, the cytoplasm contains sufficient nuclear factors to support viral RNA synthesis because of nucleus-cytoplasmic shuttling of these proteins.

Another point of note is that viral RNA synthesis usually takes place on or in distinct subcellular compartments, such as membrane-associated vesicles shown for poliovirus (Egger *et al.,* 1996; Schlegel *et al.,* 1996) and endoplasmic reticulum for BMV (Restrepo-Hartwig and Ahlquist, 1996); however, some of the cellular proteins involved in viral RNA replication (mostly RNA-processing or translation factors) are not in the vicinity of these compartments in normal cells. Thus, the association of these proteins with viral RNAs or polymerases does not occur coincidentally, but likely requires active juxtapositioning between the viral RNA synthetic machinery and the RNA-processing machinery or translation apparatus in virus-infected cells. This consideration suggests that RNA viruses may actively subvert normal cellular RNA processing or translation machineries for viral RNA synthesis. A particularly tantalizing piece of evidence for this subversion is the association of hnRNP A1 and PTB with the minus and plus strand, respectively, of mouse hepatitis virus leader RNA (Li *et al.,* 1997, and unpublished observations). The joining of the plus- and minus-strand leader sequences is a critical step in MHV RNA synthesis (Lai and Cavanagh, 1997; Lai *et al.,* 1994). Interestingly, these two proteins are associated with each other in pre-mRNA splicing complexes in normal cells (Bothwell *et al.,* 1991). Thus, MHV may subvert the entire or partial splicing complex for its RNA synthesis. These two proteins also bind to HIV and HTLV LTR sequences. Similar considerations may apply to translation factors. Some translation factors are associated with endoplasmic reticulum, where RNA synthesis of some viruses takes place; thus, the participation of translation factors in viral RNA synthesis may be logistically convenient. In all of the viruses discussed above, translation factors appear to be directly linked to viral RNA synthesis but not to viral translation, as one would have guessed otherwise. This finding suggests that it is not translation per se that is required for RNA synthesis but, rather, the translation machinery that is subverted for viral RNA synthesis. However, the translatability of viral RNA often enhances viral RNA replication (Liao and Lai, 1995; Novak and Kirkegaard, 1994; van der Most *et al.,* 1995; White *et al.,* 1992), and several RNA viruses contain tRNA-like structures at the 3′ end of the viral genome (Haenni *et al.,* 1982; Pilipenko *et al.,* 1992), further suggesting that translation factors actively participate in viral RNA replication and transcription.

The association of the pol III transcript-binding protein, La antigen, with several viral RNAs suggests that this class of proteins may also be involved in viral RNA synthesis. It is noteworthy that several RNA viruses, e.g., BMV, have pol III promoter-like sequences as their transcription initiation sequences (Pogue and Hall, 1992). Pol III transcripts are often associated with snRNPs, raising the possibility that some snRNPs may also participate in viral RNA synthesis.

RNA replication or transcription may involve the formation of RNA–protein complexes similar to the DNA-dependent RNA transcription complexes

The cellular proteins that bind to viral RNA may serve to bring various regions of a viral RNA template together to form transcription or replication complexes. Such RNA–protein complexes may help recruit and stabilize the RdRP to the initiation sites of viral RNA synthesis. Increasing evidence suggests that the *cis*-acting signals for viral RNA replication or transcription often consist of multiple discontiguous sequences on the viral RNA. Particularly, in many cases, there appears to be cross-talk between the 5′ and 3′ ends of viral RNAs, so that the 3′ end sequence often can regulate RNA synthesis or translation initiated from the 5′ end of the RNA. For example, the 3′-end sequences of BMV (Lahser *et al.,* 1993) and TMV (Leathers *et al.,* 1993) RNAs can influence plus-strand synthesis (which starts from the 5′ end of the RNA). The BMV minus-strand RNA synthesis, which starts from the 3′ end of the RNA, requires a *cis*-acting enhancer sequence upstream of the subgenomic RNA promoter (Pogue and Hall, 1992; Quadt *et al.,* 1995). A similar long-distance interaction between various RNA regions is also required to initiate RNA synthesis of alfalfa mosaic virus and L-A virus, a double-stranded RNA virus of yeast (Esteban *et al.,* 1989; van der Vossen *et al.,* 1995). In addition, the 3′ end of the MHV RNA can regulate mRNA synthesis starting from an upstream internal promoter (Lin *et al.,* 1996). Finally, there is clearly an interaction between the leader and intergenic sequences which regulate the synthesis of coronavirus subgenomic mRNAs (Lai and Cavanagh, 1997; Zhang *et al.,* 1994). Why is such an interaction necessary for the RNA replication and transcription of most viruses? One can surmise that it ensures that the plus- and minus-strand RNA syntheses are efficiently linked. However, this does not seem to be a necessary requirement for RNA viruses; in fact, most viruses synthesize the two opposite RNA strands asymmetrically. A more persuasive argument is that these 5′- and 3′-end interactions provide an advantage for viruses because only intact viral RNAs can be used as templates for RNA replication and transcription.

The interactions between 5′ and 3′ ends and other internal viral RNA sequences may involve complementary nucleotides of RNA, as do the 5′ and 3′ ends of VSV and influenza virus RNAs (Luo *et al.,* 1991; Wertz *et al.,* 1994). Influenza viral RdRP can then bind directly to the duplex structure formed between the two ends of viral RNA (Tiley *et al.,* 1994); even in these cases, the participation of cellular factors has not been ruled out (Shimizu

et al., 1994). Alternatively, where no apparent sequence complementarity exists between the 5′ and 3′ ends, RNA–protein and protein–protein interactions must be involved. The cellular proteins discussed so far may fulfill such a function. Indeed, several cellular proteins shown to bind different regions of a viral RNA have the ability to interact with each other (e.g., between PTB and hnRNP A1 for MHV), thus allowing different RNA regions to interact. For some viruses, e.g., hepatitis A virus (Kusov *et al.,* 1996), the same set of cellular proteins bind to both the 5′ and 3′ ends, potentially allowing the 5′ and 3′-end interactions through a single protein. These cellular proteins may serve as a platform on which other proteins, both viral and cellular, subsequently bind. Since most of the viral RdRPs do not appear to bind directly to the *cis*-acting regulatory or promoter sequences on the RNA, their ability to initiate RNA synthesis at specific sites probably depends on their interactions with the cellular proteins that bind directly to the viral RNA template. Such interactions may take place between the viral RdRP and cellular factors to form an RdRP complex without the RNA template (Table 1, mode I), a la a preformed transcription complex in DNA-dependent RNA transcription, or occur after the cellular factors have already docked with the RNA template (Table 1, mode II). As a result, viral RdRPs and cellular factors cooperate to form transcription or replication complexes on the viral RNA. These cellular factors will assume roles akin to those of sigma factors or TATA-binding protein and transcription factors in DNA-dependent RNA transcription (Fig. 1). Thus, RNA viruses may assemble an RNA transcription or replication complex in a similar fashion to DNA-dependent RNA transcription complexes. The formation of such ribonucleoprotein complexes will bring discontiguous regions of viral RNA together, in a manner similar to the interaction between enhancer and promoter elements in DNA-dependent RNA synthesis (Fig. 1). Assembly of such multiprotein complexes may serve as a docking site to facilitate and/or enhance the recruitment of the RdRP to the promoter region. The possible presence of RNA enhancer elements for regulating RNA synthesis has been proposed for MHV and BMV RNA synthesis (Liao and Lai, 1994; Quadt *et al.,* 1995). Thus, one end of the viral RNA template serves as an enhancer-like element, while the other serves as the promoter at various steps of viral RNA synthesis. Many transcription factors likely are components of cellular RNA-processing pathways or translation machinery. These factors interact with each other and viral polymerases to effect RNA transcription or replication.

Perspectives

The discussion here suggests that cellular factors are heavily involved in viral RNA replication and transcription. In addition to the proteins identified so far, many partially purified RdRPs contain additional unidentified cellular proteins, and various viral RNAs bind still many more yet-to-be characterized cellular factors. Characterization of these proteins will require extensive molecular biological and biochemical analysis. Recent studies showed that some RNA viruses, e.g., BMV and flock house virus, can replicate in yeast (Janda and Ahlquist, 1993; Price *et al.,* 1996). Yeast mutants which cannot support viral RNA replication or transcription have been isolated, indicating that viral RNA synthesis, indeed, requires cellular factors (Ishikawa *et al.,* 1997). Although viral replication in a heterologous host may demand additional factors not seen in the homologous natural host, this experimental system, nevertheless, offers an elegant and powerful approach to genetic analysis of cellular factors involved in viral RNA synthesis.

In conclusion, viral RNA-dependent RNA synthesis may be as complex as DNA-dependent RNA transcription, involving many RNA–protein and protein–protein interactions between viral RNA or proteins and cellular factors. Thus, host cells are not simply bystanders, but are active participants in the process of viral RNA transcription and replication. Identification of these proteins will hasten our efforts to unlock many of the mysteries of viral RNA replication and transcription.

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